Serial No. : 09/943,857
Filed : August 31, 2001

Page : 2 of 27

#### Amendments to the Specification:

Please replace the paragraph beginning at page 3, line 24 with the following amended paragraph:

-- The "percent identity" (or "percent homology") of two amino acid sequences or of two nucleic acids can be determined using the algorithm of Thompson et al. (CLUSTAL W, 1994 Nucleic Acids Res. 22: 4673-4680). An amino acid sequence or a nucleotide sequence can also be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the algorithm of Karlin and Altschul (1990 Proc. Natl. Acad. Sci. USA 87: 2264-2268), modified as in Karlin and Altschul (1993 Proc. Natl. Acad. Sci. USA 90: 5873-5877). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990 J. Mol. Biol. 215: 403-410). BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12. BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3. Where gaps exist between two sequences, Gapped BLAST is utilized as described in Altschul et al. (1997 Nucleic Acids Res. 25: 3389-3402). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used. See www.ncbi.nlm.nih.gov/.--

Please replace the three paragraphs beginning at page 16, line 29 with the following amended paragraphs:

-- Transformation of plasmid DNAs into P. pastoris. Plasmid DNA (10 μg) harboring the engineered lipase gene was digested with EcoRV in a total volume of 20 μL for 2 h. Linearized plasmid was transformed into P. pastoris by the electroporation method. High voltage pulses (1.5 kV) were delivered to 100 μL samples in 0.2 cm electrode gap cuvettes (Bio-Rad Laboratories) by using a GENE PULSER electrophoration apparatus with the Pulse Controller (Bio-Rad Laboratories). Individual colonies of transformants were pitched and patched on tributyrin-emulsion YPD plates. The lipase-secreting transformants were identified by the clear

Serial No. : 09/943,857
Filed : August 31, 2001

Page : 3 of 27

zone on the opaque tributyrin emulsion. P. pastoris transformed with pGAPZ $\alpha$ C was used as a negative control.

Purification of recombinant LIP2. The culture medium from P. pastoris was concentrated by ultrafiltration on a 30,000 molecular weight cut-off membrane. These samples were then applied onto a HIPREP 16/10 Octyl FF chromatographic column (Pharmacia Biotech). The column was washed with 5 column volumes of TE buffer plus 1 mM CHAPS and then 4 mM CHAPS. Bound proteins were then eluted with 5 column volumes of TE buffer containing 30 mM CHAPS. The eluted materials were dialyzed against TE buffer.

The eluted proteins were then applied to a HIPREP 16/10 Q XL column (Pharmacia Biotech) equilibrated with TE buffer and the proteins were eluted using a linear gradient of 0 to 300 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> over 5 column volumes. Protein concentrations in the fractions were measured with the Bio-Rad assay kit and esterase activity was determined using *p*-nitrophenyl butyrate as a substrate. Purified proteins were stored in a storage buffer (60 mM KCl, 10 mM Tris-HCl, 1.25 mM EDTA, 1% Triton X-100, and 17% glycerol, pH 7.5) at -20°C.--

Please replace the paragraph beginning at page 5, line 24 with the following amended paragraph:

-- Set forth below are the mutant nucleic acid sequences of *C. rugosa* lipase 2, *C. rugosa* lipase 3, *C. rugosa* lipase 4, *C. rugosa* lipase 5, and *C. rugosa* lipase 8, wherein all the CTG codons corresponding to serine in the wild-type DNA have been substituted with one of the six universal serine codons (i.e., TCT, TCC, TCA, TCG, AGT, or AGC). The mutated nucleotides are represented in black background. Also shown are the encoded amino acid sequences. The introduced N-terminal peptide (i.e., SMNSRGPAGRLGS) is underlined. Mutated amino acid residues are rendered in bold.

## Mutant C. rugosa lipase 2

TCGATGAATTCACGTGGCCCAGCCGGCCGTCTCGGATCGGTACCCACCGCCACGCTCGCC 60

S M N S R G P A G R L G S V P T A T L A 20

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 4 of 27

AACGGCGACACCATCACCGGTCTCAACGCCATTGTCAACGAAAAGTTTCTCGGCATACCG 120 G D T I T G L N A I V N E K F L G I P TTTGCCGAGCCGCCGTGGGCAGCCTCCGCTTCAAGCCGCCCGTGCCGTACTCGGCGTCG 180 E P P V G S L R F K P P V P Y S A S G Q Q F T S Y G P S C M Q M N P M G TCGTTTGAGGACACACTTCCCAAGAATGCGCTTCACTTGAGTTCCCAAGATCTTC 300 FEDTLPKNA**LD**LVLQSKIF 100 CAAGTGGTGCTTCCCAACGACGACGACTGTCTCACCATCAACGTGATCCGGCCGCCCGGC 360 O V V L P N D E D C L T I N V I R P P G ACCAGGGCCAGTGCTGGTCTCCCGGTGATGCTCTGGATCTTTGGCGGTGGGTTTGAGCTT 420 R A S A G L P V M L W I F G G G F E L GGCGGCTCCAGCCTCTTTCCAGGAGACCAGATGGTGGCCAAGAGCGTGCTCATGGGTAAA 480 G G S S L F P G D Q M V A K S V L M G K 160 V I H V S M N Y R V A S W G F L A G P GACATCCAGAACGAAGGCAGCGGGAACGCCGGCTTGCATGACCAGCGCTTGGCCATGCAG 600 Q N E G S G N A G L H D Q R L A M Q TGGGTGGCGGACAACATTGCTGGGTTTGGCGGCGACCCGAGCAAGGTGACCATATACGGC 660 V A D N I A G F G G D P S K V T I Y G GAGTCTGCGGGCAGCATGTCGACGTTTGTGCACCTTGTGTGGAACGACGGCGACAACACG 720 E S A G S M S T F V H L V W N D G D N T TACAACGGCAAGCCGTTGTTCCGCGCCGCCATCATGCAGTCTGGCTGCATGGTGCCGTCT 780 Y N G K P L F R A A I M Q S G C M V P S GACCCGGTGGACGCACGTACGCCACCGAGATCTACAACCAGGTGGTGGCGTCTGCCGGG 840 D P V D G T Y G T E I Y N Q V V A S A G 280

Serial No. : 09/943,857
Filed : August 31, 2001

Page : 5 of 27

TGTGGCAGTGCCAGCGACAAGCTCGCGTGCTTGCGCGGCCTTTCTCAGGACACGTTGTAC 900 C G S A S D K L A C L R G L S Q D T L Y CAGGCCACGACGCCCGGCGTGTTGGCGTACCCGTCGTTGCGGTTGTCTTATCTC 960 O A T S D T P G V L A Y P S L R L S Y L CCGCGGCCCGACGGCACCTTCATCACCGACGACATGTATGCCTTGGTGCGGGACGGCAAG 1020 PRPDGTFITDDMYALVRDGK340 Y A H V P V I I G D Q N D E G T L F G L TCTTCTTTGAACGTGACCACAGATGCTCAGGCACGGGCGTACTTCAAGCAGTCTTTCATC 1140 LNVTTDAOARAYFKOSF CACGCCAGCGATGCGGAGATCGACACGTTGATGGCGGCGTACACCAGCGACATCACCCAG 1200 H A S D A E I D T L M A A Y T S D I T Q GGTTCTCCGTTCGACACCGGCATCTTCAATGCCATCACCCCGCAGTTCAAACGGATCTCT 1260 G S P F D T G I F N A I T P Q F K R I S GCGTTGCTTGGCGACCTTGCGTTCACGCTTGCGCGTCGCTACTTCCTCAACTACTACCAG 1320 A L L G D L A F T L A R R Y F L N Y Y Q GGCGGCACCAAGTACTCGTTCTAAGCAGCTTTCTGGGTTGCCCGTCTTGGGCACC 1380 G G T K Y S F L S K O L S G L P V L G TTCCACGGCAACGACATCATCTGGCAGGACTACTTGGTGGGCAGCGGCAGTGTGATCTAC 1440 H G N D I I W O D Y L V G S G S V I Y AACAACGCGTTCATTGCGTTTGCCAACGACCTCGACCCGAACAAGGCGGGCTTGTGGACC 1500 N N A F I A F A N D L D P N K A G L W T AACTGGCCCACGTACACCAGCAGCTCTCAGTCTGGCAACAACTTGATGCAGATCAACGGC 1560 P T Y T S S S Q S G N N L M Q I N G TTGGGGTTGTACACCGGCAAGGACAACTTCCGCCCGGATGCGTACAGCGCCCTCTTTTCC 1620 LGLYTGKDNFRPDAYSALFS 540

Serial No.: 09/943,857
Filed: August 31, 2001

Page : 6 of 27

AACCCGCCGTCTTTCTTTGTG (SEQ ID NO:1)

N P P S F F V (SEQ ID NO:2)

547

1641

# Mutant C. rugosa lipase 3

TCG	GATGAATTCACGTGGCCCAGCCGGCCGTCTCGGATCG <mark>GTA</mark> CCCACCGCCAAGCTCGC														GCC	60				
S	М	N	S	R	G	Р	Α	G	R	L	G	S	v	P	T	Α	K	L	A	20
AAC	${\tt AACGGCGACACCATCACCGGTCTCAACGCCATCATCAACGAGGCGTTCCTCGGCATTCCC}$															120				
N	G	D	Т	I	T	G	L	N	Α	I	I	N	E	Α	F	L	G	I	P	40
ттт	$\tt TTTGCCGAGCCGCTGGGCAACCTCCGCTTCAAGGACCCTGTGCCGTACTCTGGCTCG$															180				
F	A	E	P	P	V	G	N	L	R	F	K	D	P	V	P	Y	S	G	s	60
СТС	$\tt CTCAACGGCCAGAAGTT{\color{red}C}ACT{\color{red}TCT}TACGGCCCG{\color{red}TCT}TGCATGCAGCAGAACCCCGAGGGC$															240				
L	N	G	Q	K	F	Т	s	Y	G	P	s	С	M	Q	Q	N	P	E	G	80
ACG	ACGTTTGAAGAGAACCTTGGCAAGACGGCACTCGACTTGGTGATGCAGTCCAAGGTGTTC															TTC	300			
Т	F	E	E	N	L	G	K	T	Α	L	D	L	V	M	Q	s	K	V	F	100
CAG	CAGGCGGTGCTTCCCCAGAGTGAGGACTGCCTCACCATCAACGTGGTGCGGCCGCCGGGC															GGC	360			
Q	A	V	L	P	Q	S	E	D	С	L	Т	I	N	V	V	R	Р	P	G	120
ACC	ACCAAGGCGGCCCAACCTCCCGGTCATGCTCTGGATCTTTGGCGGTGGGTTTGAGATC															ATC	420			
Т	K	Α	G	Α	N	L	P	V	M	L	W	I	F	G	G	G	F	E	I	140
GGC	GGCAGCCCCACCATCTTCCCTCCCGCCCAGATGGTCACCAAGAGTGTGCTCATGGGCAAG															AAG	480			
G	s	Р	Т	I	F	P	P	Α	Q	M	V	Т	K	s	V	L	M	G	K	160
CAC	CACATCATCCACGTGGCCGTCAACTACCGTGTTGCCTCGTGGGGGTTCTTGGCTGGTGAT															GAT	540			
н	I	I	Н	V	Α	V	N	Y	R	V	Α	s	W	G	F	L	Α	G	D	180
GAC	ATC	AAG	GCC	GAG	GGC	AGC	GGG	AAC	GCC	GGC	ттс	AAG	GAC	CAG	CGI	'TTG	GGC	ATG	CAG	600
D	I	K	A	E	G	s	G	N	Α	G	L	K	D	Q	R	L	G	M	Q	200
TGG	GTG	GCA	GAC	AAC	TTA	'GCC	GGG	TTC	GGC	GGC	GAC	CCG	AGC	AAG	GTG	ACT	ATC	ттт	GGC	660

Applicant: Jei-Fu Shaw et al.

Serial No.: 09/943,857 Filed: August 31, 2001

Page : 7 of 27

V A D N I A G F G G D P S K V T I F G 220 GAGTCTGCGGGCAGCATGTCCGTGTTGTGCCACCTCATCTGGAACGACGGCGACAACACG 720 S A G S M S V L C H L I W N D G D N T TACAAGGGCAAGCCGTTGTTCCGCGCGGGCATCATGCAGTCTGGAGCCATGGTGCCGTCT 780 K G K P L F R A G I M Q S G A M GACCCGGTGGACGCACGTACGGCAACGAGATCTACGACCTCTTTGTCTCGAGTGCTGGC 840 D P V D G T Y G N E I Y D L F V S S A G TGTGGCAGCGCCAGCGACAAGCTCGCGTGCTTGCGCAGTGCGTCTAGCGACACCTTGCTC 900 C G S A S D K L A C L R S A S S D T L L GATGCCACCAACACCTCCTGGGTTCTTGGCGTACTCCTCGTTGCGGTTGTCTTATCTC 960 D A T N N T P G F L A Y S S L R L S Y L CCECGGCCGACGCAAGAACATCACCGATGACATGTACAAGTTGGTGCGCGACGGCAAG 1020 R P D G K N I T D D M Y K L V R D G K TATGCAAGCGTTCCCGTGATCATTGGCGACCAGAACGACGAGGGCACCATCTTTGGGCTC 1080 Y A S V P V I I G D Q N D E G T I F G L TCTTCTTTGAACGTGACCACGAATGCTCAGGCCCGTGCTTACTTCAAGCAGTCTTTCATC 1140 S L N V T T N A Q A R A Y F K Q S F CACGCCAGCGACGCGGAGATCGACACCTTGATGGCGGCGTACCCCCAGGACATCACCCAG 1200 H A S D A E I D T L M A A Y P Q D I T GGTTCTCCGTTCGACACGGGTGTTCTCAACGCCCTCACCCCGCAGTTCAAGAGAATCTCT 1260 SPFDTG**VL**NA**L**TPQFKRIS 420 GCGGTGCTCGGCGACCTTGCATTCATCCACGCCCGCCGCTACTTCCTCAACCACTTCCAG 1320 A V L G D L A F I H A R R Y F L N H F Q GGCGGCACCAAGTACTCGTTCCTCTAAGCAGCTCTCTGGGTTGCCAATCATGGGCACC 1380 K Y S F L S K Q L S G L P I M G T TTCCATGCCAACGACATTGTGTGGCAGGACTACTTGTTGGGAAGCGGCAGCGTCATCTAC 1440

Serial No.: 09/943,857
Filed: August 31, 2001

Page : 8 of 27

F H A N D I V W Q D Y L L G S G S V I Y 480

AACAACGCGTTTATCGCGTTCGCCACCGACTTGGACCCCAACACCGCGGGGTTGTTGGTG 1500

N N A F I A F A T D L D P N T A G L L V 500

AACTGGCCCAAGTACACCAGCAGCTCTCAGTCTGGCAACAACTTGATGATGATCAACGCC 1560

N W P K Y T S S S Q S G N N L M M I N A 520

TTGGGCTTGTACACCGGCAAGGACAACTTCCGCACCGCTGGCTACGACGCGTTGATGACC 1620

L G L Y T G K D N F R T A G Y D A L M T 540

AACCCGTCTTCTTTCTTTGTG (SEQ ID NO:3) 1641

## Mutant C. rugosa lipase 4

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 9 of 27

G G S S L F P P A Q M I T A S V L M G K 160 CCCATCATCCACGTGAGCATGAACTACCGCGTTGCTTCGTGGGGGGTTCTTGGCTGGTCCA 540 I I H V S M N Y R V A S W G F L A G P GACATCAAGGCCGAGGGCAGCGGGAACGCCGGTTTGCACGACCAACGCTTGGGTTTGCAG 600 D I K A E G S G N A G L H D Q R L G L Q TGGGTGGCGGACACATTGCCGGGTTCGGCGGCGACCCGTCCAAGGTGACCATCTTTGGT 660 W V A D N I A G F G G D P S K V T I F G 220 GAGTCGGCGGCAGCATGTCGGTAATGTGTCAGCTCCTCTGGAACGACGGCGACAACACG 720 E S A G S M S V M C Q L L W N D G D N T TACAACGGCAAGCCGTTGTTCCGTGCCGCCATCATGCAGTCTGGGGGCCATGGTGCCGTCG 780 Y N G K P L F R A A I M Q S G A M V P GACCCGGTGGATGGGCCCTACGGCACGCAGATCTACGACCAGGTGGTTGCTTCAGCCGGC 840 TGTGGCAGTGCCAGCGACAAGCTCGCGTGCTTGCGCAGCATCTCGAACGACAAACTCTTC 900 C G S A S D K L A C L R S I S N D K L F CAGGCCACCAGCGACACTCCGGGGGCCTTGGCGTACCCCTCGTTGCGGTTGTCGTTTCTC 960 Q A T S D T P G A L A Y P S L R L S F L CCGCGGCCCGACGCACCTTCATCACCGATGACATGTTCAAGTTGGTGCGCGACGGCAAG 1020 R P D G T F I T D D M F K L V R D G K TGTGCCAACGTTCCGGTGATCATTGGCGACCAGAACGACGAGGGCACAGTGTTTGCGTTG 1080 C A N V P V I I G D Q N D E G T V F A L TCCAGCTTGAACGTGACTACGGATGCTCAGGCACGCCAGTACTTCAAGGAAAGCTTCATC 1140 S L N V T T D A Q A R Q Y F K E S F I CACGCCAGCGACGCGGAGATCGACACCTTGATGGCGGCGTACCCCAGCGACATCACCCAG 1200 H A S D A E I D T L M A A Y P S D I T Q GGTAGTCCGTTCGACACCGGCATCTTCAACGCCATCACCCCGCAGTTCAAACGGATTGCA 1260

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 10 of 27

G S P F D T G I F N A I T P Q F K R I A 420 GCGGTGCTTGGTGACCTTGCGTTCACTCTCCCCCGGCGCTACTTCCTCAACCACTTCCAG 1320 A V L G D L A F T L P R R Y F L N H F Q 440 GGCGGCACCAAGTACTCGTTCCTCTCGAAGCAGCTTAGTGGGTTGCCGGTGATTGGCACC 1380 G G T K Y S F L S K O L S G L P V I G T CACCACGCCAACGACATTGTGTGGCAGGACTTTTTGGTGAGCCACAGCAGCGCCGTGTAC 1440 H H A N D I V W Q D F L V S H S S A V Y 480 AACAACGCGTTTATTGCCTTTGCCAACGACCTCGACCCGAACAAGGCCGGTTTGCTTGTG 1500 N A F I A F A N D L D P N K A G L L V AACTGGCCCAAGTACACCAGCAGCTCTCAGTCAGGCAACAACTTGTTGCAGATCAACGCC 1560 N W P K Y T S S S Q S G N N L L Q I N A TTGGGCTTGTACACCGGCAAGGACAACTTCCGCACCGCTGGCTACGACGCGTTGTTTACC 1620 L G L Y T G K D N F R T A G Y D A L F T AACCCGTCTTCTTTCTTTGTG (SEQ ID NO:5) 1641 547 N P S S F F V (SEQ ID NO:6)

### Mutant C. rugosa lipase 5

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 11 of 27

T Y E E N L P K V A L D L V M O S K V F CAGGCTGTTCTCCCCAACAGCGAGGACTGCCTCACCATCAACGTGGTGCGGCCGCCGGGC 360 O A V L P N S E D C L T I N V V R P P G ACCAAGGCGGGCGCCAACCTCCCGGTCATGCTCTGGATCTTTGGCGGTTGGGTTTGAGATC 420 K A G A N L P V M L W I F G G G F E GGCAGCCCCACCATCTTCCCTCCCGCTCAGATGGTCTCCAAGAGTGTGCTCATGGGCGAG 480 G S P T I F P P A Q M V S K S V L M G E CCCATCATCCACGTGGCCGTCAACTACCGCTTGGCGTCCTTTGGTTTCTTGGCCGGTCCG 540 I I H V A V N Y R L A S F G F L A G P GACATCAAGGCCGAGGGCAGCTCCAATGCCGGCCTCAAGGACCAGCGCTTGGGCATGCAG 600 I K A E G S S N A G L K D O R L G M O TGGGTGGCAGACACATTGCCGGGTTCGGCGGCGACCCGAGCAAGGTGACCATCTTTGGC 660 V A D N I A G F G G D P S K V T I F G GAGTCTGCGGGCAGCATGTCCGTGTTGTGCCACCTTCTCTGGAATGGCGGCGACAACACG 720 E S A G S M S V L C H L L W N G G D N T TACAAGGGCAAGCCGTTGTTCCGCGCGGGCATCATGCAGTCTGGAGCCATGGTGCCGTCT Y K G K P L F R A G I M Q S G A M V P GACCCGGTGGACGCACCTATGGAGCCCAAATCTATGACACGTTGGTGGCTTCTACGGGC 840 P V D G T Y G A Q I Y D T L V A S T TGCAGCAGTGCCAGCAACAAGCTTGCGTGCTTGCGTGGTCTTTCTACTCAGGCATTGCTC 900 S S A S N K L A C L R G L S T Q A L GATGCCACCAACGACACCCCTGGGTTCTTGTCGTACACCTCGTTGCGGTTGTCTTATCTC 960 D A T N D T P G F L S Y T S L R L S Y L CCECGCCCGACGCCCAACATCACCGATGACATGTACAAGTTGGTACGCGACGCAAG 1020 D G A N I T D D M Y K L V R D G K TATGCAAGCGTTCCCGTGATCATTGGCGACCAGAACGACGAGGGCTTCTTGTTTGATCTC 1080

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 12 of 27

Y A S V P V I I G D Q N D E G F L F D L 360 TCTTCTTTGAACACCACCACCGAGGCCGACGCCGAGGCATACCTCAGAAAGTCTTTCATC 1140 S S L N T T T E A D A E A Y L R K S F I CACGCCACCGACGCCGATATCACCGCATTGAAGGCGGCGTACCCCAGCGATGTCACCCAG 1200 H A T D A D I T A L K A A Y P S D V T Q GGTTCTCCGTTCGACACGGGCATTCTCAACGCCCTTACACCCCAGCTCAAGCGGATCAAT 1260 G S P F D T G I L N A L T P Q L K R I N 420 GCTGTGCTTGGCGACCTCACCTTTACCCTCTCGCGCCGCTACTTCCTCAACCACTACACC 1320 A V L G D L T F T L S R R Y F L N H Y T GGTGGTCCCAAGTACTCGTTCCTCTAAGCAGCTTTCTGGGTTGCCCATTCTCGGTACG 1380 G G P K Y S F L S K O L S G L P I L G T TTCCACGCGAACGACATTGTGTGGCAGCACTTTTTGTTGGGCAGCGGCAGCGTCATCTAC 1440 F H A N D I V W Q H F L L G S G S V I Y AACAACGCGTTCATCGCGTTTGCCACCGACTTGGACCCCAACACCGCGGGCTTGTCTGTG 1500 N N A F I A F A T D L D P N T A G L S V 500 CAGTGGCCCAAGTACACCAGCAGCTCTCAGGCGGGGGACAACTTGATGCAGATCAGTGCC 1560 Q W P K Y T S S S Q A G D N L M Q I S A L G L Y T G K D N F R T A G Y N A L F A 540 GACCCGTCTCACTTTTTCGTG (SEQ ID NO:7) 1641 547 D P S H F F V (SEQ ID NO:8)

#### Mutant C. rugosa lipase 8

TCGATGAATTCACGTGGCCCAGCCGGCCGTCTCGGATCGGTACCCACTGCCACGTCGCC 60

S M N S R G P A G R L G S V P T A T L A 20

AACGGCGACACCATCACCGGTCTCAACGCCATCATCAACGAGGCGTTCCTCGGCATTCCC 120

N G D T I T G L N A I I N E A F L G I P 40

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 13 of 27

TTTGCCGAGCCGCCGGTGGGCAACCTCCGCTTCAAGGACCCCGTGCCGTACTCCGGCTCG 180 FAEPPVGNLRFKDPVPYSGS D G Q K F T S Y G P S C M Q Q N P E ACCTACGAGGAGAACCTCCCCAAGGCAGCGCTCGACTTGGTGATGCAGTCCAAGGTGTTT 300 Y E E N L P K A A L D L V M Q S K V F GAGGCGGTGTCTCCGTCTAGCGAGGACTGTCTCACCATCAACGTGGTGCGGCCGCCGGGC 360 E A V S P S S E D C L T I N V V R P P G ACCAAGGCGGGTGCCAACCTCCCGGTGATGCTCTGGATCTTTGGCGGCGGGTTTGAGGTG 420 K A G A N L P V M L W I F G G G F E V GGTGGCACCAGCACCTTCCCTCCCGCCCAGATGATCACCAAGAGCATTGCCATGGGCAAG 480 G G T S T F P P A Q M I T K S I A M G K P I I H V S V N Y R V S S W G F L A G D GAGATCAAGGCCGAGGGCAGTGCCAACGCCGGTTTGAAGGACCAGCGCATGGGCATGCAG 600 I K A E G S A N A G L K D Q R M G M Q TGGGTGGCGGACAACATTGCGGCGTTTGGCGGCGACCCGACCAAGGTGACCATCTTTGGC 660 V A D N I A A F G G D P T K V T I F G GAGTCTGCGGGCAGCATGTCGGTCATGTGCCACATTCTCTGGAACGACGGCGACAACACG 720 A G S M S V M C H I L W N D G D N T TACAAGGGCAAGCCGCTCTTCCGCGGGGCATCATGCAGTCTGGGGGCCATGGTACCGTCG 780 Y K G K P L F R A G I M O S G A M V P S GACGCGTGGACGGCTCTACGGCAACGAGATCTTTGACCTCTTGGCGTCGGACGCGGC 840 DAVDG**V**YGNEIFDLLAS**D**AG TGCGGCAGCGCCAGCGACAAGCTTGCGTGCTTGCGCGGTGTGTCTAGCGACACGTTGGAG 900  $\begin{smallmatrix} C & G & S & A & S & D & K & L & A & C & L & R & G & V & S & S & D & T & L & E \\ \end{smallmatrix}$ 

Applicant: Jei-Fu Shaw et al.

Serial No.: 09/943,857 Filed: August 31, 2001

Page : 14 of 27

GACGCCACCAACACCCCTGGGTTCTTGGCGTACTCCTCGTTGCGGTTGTCTTATCTC 960 D A T N. N T P G F L A Y S S L R L S Y L 320 CCCCGCCGCCGACGCGTGAACATCACCGACGACATGTTTGCCTTGGTCCGCGAGGGCAAG 1020 PRPDGVNITDDM**F**ALVREGK 340 TATGCMACCGTTCCTGTGATCATCGGCGACCAGAACGACGAGGGCACCTTCTTTGGCACC 1080 Y A S V P V I I G D Q N D E G T F F G T TCTTCTTTGAACGTGACCACGGATGCCGAGGCCCGCCAGTACTTCACGCAGTCTTTTGTC 1140 S S L N V T T D A E A R Q Y F T Q S F V CACGCCAGCGACGCGGAGETCGACACGTTGATGACGGCGTACCCCCCAGGACATCACCCAG 1200 HASDAE L D T L M T A Y P Q D I GGTTCTCCGTTCGACACGGGTCTTCTCAACGCCCTCACCCCGCAGTTCAAGAGAATCTCT 1260 G S P F D T G V L N A L T P Q F K R I S 420 A V L G D L A F I H A R R Y F L N H Y T 440 GGCGGCACCAAGTACTCATTCCTCTAAGCAGCTCTCTGGCTTGCCGGTGCTCGGAACG 1380 G G T K Y S F L S K Q L S G L P V L G T H S N D I V F Q D Y L L G S G S L I Y 480 AACAACGCGTTCATTGCGTTTGCCACGGACTTGGACCCCAACACCGCGGGGTTGTTGGTG 1500 N N A F I A F A T D L D P N T A G L L V 500 AAGTGGCCCGAGTACACCAGCAGCTCTCAGTCTGGCAACAACTTGATGATGATCAACGCC 1560 K W P E Y T S S Q S G N N L M M I N A 520 TTGGGCTTGTACACCGGCAAGGACAACTCCCGCACCGCCGGCTACGACGCGTTGTTCTCC 1620 LGLYTGKDN**S**RTAGYDALFS 540 AACCCGCCGTCTTTCTTTGTG (SEQ ID NO:9) 1641 N P P S F F V (SEQ ID NO:10) 547 --

Applicant: Jei-Fu Shaw et al. Serial No.: 09/943,857 Filed: August 31, 2001

Page : 15 of 27

Please replace the paragraph beginning at page 14, line 4 with the following amended paragraph:

-- The polypeptide having the amino acid sequence of SEQ ID NO:2 differs from the wild-type *C. rugosa* lipase 2 by a N-terminal peptide (i.e., SMNSRGPAGRLGS) and 4 amino acids (i.e., A14V; T48S; R91L; H92D). The polypeptide having the amino acid sequence of SEQ ID NO:4 differs from the wild-type *C. rugosa* lipase 3 by the N-terminal peptide and 5 amino acids (i.e., A14V; P161H; I408V; F409L; I412L). The polypeptide having the amino acid sequence of SEQ ID NO:6 differs from the wild-type *C. rugosa* lipase 4 by the N-terminal peptide and 1 amino acid (i.e., A14V). The polypeptide having the amino acid sequence of SEQ ID NO:8 differs from the wild-type *C. rugosa* lipase 5 by the N-terminal peptide and 5 amino acids (i.e., A14V; K160E; T269A; G359D; S505Y). The polypeptide having the amino acid sequence of SEQ ID NO:10 differs from the wild-type *C. rugosa* lipase 1 by the N-terminal peptide and 17 amino acids (i.e., A14V; L197M; I266V; N278D; Y333F; N343S; I344V; O370E; E373Q; K376T; I387L; G396Q; I408V; G427A; T429I; L430H; F530S).--